

FORM-PTO-1390 (Rev. 12-29-99)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER 012627-022	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) <div style="font-size: 24pt; font-weight: bold;">09/868502</div>	
INTERNATIONAL APPLICATION NO. PCT/DE99/04052		INTERNATIONAL FILING DATE 17 December 1999		PRIORITY DATE CLAIMED 17 December 1998	
TITLE OF INVENTION FOAMY VIRUS VECTORS FOR EXPRESSING FOREIGN GENES IN MAMMALS AND THE USE THEREOF					
APPLICANT(S) FOR DO/EO/US Rolf M. FLUGEL; Martin LOCHLT; Robert FLOWER; Ingrid WINKLER					
<p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:</p> <ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1). <div style="margin-left: 20px;"> <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <div style="margin-left: 20px;"> <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). <input checked="" type="checkbox"/> has been transmitted by the International Bureau. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US) </div> </div> <input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)). <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <div style="margin-left: 20px;"> <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> have been transmitted by the International Bureau. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. <input type="checkbox"/> have not been made and will not be made. </div> 8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). 10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). <p>Items 11. to 16. below concern other document(s) or information included:</p> <ol style="list-style-type: none"> 11. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. <input checked="" type="checkbox"/> A FIRST preliminary amendment. <div style="margin-left: 20px;"> <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. </div> 14. <input type="checkbox"/> A substitute specification. 15. <input type="checkbox"/> A change of power of attorney and/or address letter. 16. <input type="checkbox"/> Other items or information: 					

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.50) 09/868502		INTERNATIONAL APPLICATION NO. PCT/DE99/04052		ATTORNEY'S DOCKET NUMBER 012627-022	
--	--	--	--	---	--

17. <input checked="" type="checkbox"/> The following fees are submitted:	CALCULATIONS	PTO USE ONLY			
Basic National Fee (37 CFR 1.492(a)(1)-(5)):					
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1,000.00 (960)					
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$860.00 (970)					
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$710.00 (958)					
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$690.00 (956)					
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 (962)					
ENTER APPROPRIATE BASIC FEE AMOUNT =		\$ 860.00			
Surcharge of \$130.00 (154) for furnishing the oath or declaration later than 20 <input type="checkbox"/> 30 <input type="checkbox"/> months from the earliest claimed priority date (37 CFR 1.492(e)).		\$			
Claims	Number Filed	Number Extra	Rate		
Claims	16 -20 =	0	X\$18.00 (966)	\$	--
Independent Claims	1 -3 =	0	X\$80.00 (964)	\$	--
Multiple dependent claim(s) (if applicable)			+ \$270.00 (968)	\$	
TOTAL OF ABOVE CALCULATIONS =				\$ 860.00	
Reduction for 1/2 for filing by small entity, if applicable (see below).				\$ 430.00	-
SUBTOTAL =				\$ 430.00	
Filing fee of \$130.00 (156) for furnishing the English translation later than 20 <input type="checkbox"/> 30 <input type="checkbox"/> months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
TOTAL NATIONAL FEE =				\$ 430.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 (581) per property +				\$	
TOTAL FEES ENCLOSED =				\$ 430.00	
				Amount to be:	
				refunded	\$
				charged	\$

a. ☐ Small entity status is hereby claimed.

b. ☒ A check in the amount of \$ 430.00 to cover the above fees is enclosed.

c. ☐ Please charge my Deposit Account No. 02-4800 in the amount of \$ to cover the above fees. A duplicate copy of this sheet is enclosed.

d. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Teresa Stanek Rea
BURNS, DOANE, SWECKER & MATHIS, L.L.P.
P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

SIGNATURE

 Teresa Stanek Rea
 NAME

30,427
 REGISTRATION NUMBER

K 2978

**Foamy Virus Vectors for Expressing Foreign Genes in Mammals
and the Use Thereof**

The present invention relates to retroviral vectors based on foamy viruses (FV)/spumaviruses for introducing a desired expressible DNA into mammalian cells and mammals, the retroviral vector comprising the following sequences: a first DNA sequence which corresponds to the reverse transcript of part of a feline FV (feFV) and a second DNA sequence which permits the propagation in bacteria. The present invention also relates to medicaments containing these vectors.

Retroviruses are RNA viruses in which the viral genes of a single-stranded RNA molecule are encoded. Having accessed the host cell, the viral RNA is converted via reverse transcription into a double-stranded DNA molecule. Thereafter, this DNA reaches the nucleus and integrates into the host genome as what is called a provirus which, in turn, is the template for the expression of viral genes and the synthesis of virion RNA. The viral gene products and the virion RNA combine into an intact virion which may then leave the cell again and infect new cells. In the past, it could be shown that it is possible by means of retroviruses to introduce foreign DNA into a desired organism where it is expressed. Thus, retroviruses offer themselves fundamentally as a vehicle for a gene therapy. However, the formerly achieved success was not yet satisfactory, since as a rule the retroviral vectors used for treating animals or humans only have minor efficiency. In particular the qualitatively insufficient expression limited in time of the therapeutic gene are essential limitations of the current retroviral vectors (Anderson 1998, Nature 392, 25).

Thus, the invention is substantially based on the technical

problem of providing a viral vector by means of which heterologous genes can efficiently be inserted in a desired mammal, e.g. a cat or human being, and be expressed efficiently.

The solution to this technical problem was achieved by the provision of the embodiments characterized in the claims.

It could be shown that by means of a vector based on a feline foamy virus (FeFB) it is possible to introduce desired heterologous genes efficiently into a desired mammal where they can also be expressed. The advantages of a vector based on the feline FV are presumably based on its replication mechanism which differs from that of other retroviruses. For example, FeFV have an internal promoter for the expression of the regulatory "bel" genes which are required for the replication of the virus. The "pol" proteins are expressed in addition via a spliced RNA but not as part of a "gag-pol" fusion protein. Since the FeFV induces a productive and lifelong persisting infection of cats with permanent antigen expression, the FeFV has intrinsically mechanisms to overcome the above-mentioned deficiencies of known vectors. Furthermore, spumaviruses are physically relatively stable (minimal titer reduction when stored at 4°C), they have a minor genetic variation, a high insertion capacity and are considered nonpathogenic; these are characteristics which support the use as retroviral vectors.

For the production of an exemplary vector the entire genome of feline FV (FeFV) was inserted from the nucleotide positions 17 to 3'LTR in the prokaryotic vector pAT153 in sequential steps between the EagI-(5'LTR) and the ClaI-(3'LTR) restriction sites. At the 5' end of the viral DNA insert there are in this case various inserted restriction sites. This recombinant DNA is genetically stable e.g. in *E. coli* K12. The biological activity of the recombinant FeFV DNA (expression of infectious virions) was checked after the transfection in permissive cells (CRFK) and it showed that infectious virions formed. They do not differ as regards

their gene expression from the initial isolate (non-cloned FeFV). Moreover, it could be shown that when using this viral vector in which DNA sequences were inserted which code for neutralization-relevant epitopes of the Env surface protein of a serologically distinct, genetically differing FeFV isolate, these heterologous domains were expressed in transfected cells in a functionally active way.

Thus, an embodiment of the present invention relates to a retroviral vector for introducing a desired expressible DNA into a mammalian cell, the retroviral vector comprising the following sequences: a first DNA sequence which corresponds to the reverse transcript of at least part of a feline FV genome and a second DNA sequence which permits the propagation in bacteria and thus the efficient and site-directed genetic modification.

Another constituent of the vector is optionally a desired expressible foreign-DNA/therapeutic gene.

The expression "...corresponds to the reverse transcript of at least part of an FV genome" used herein relates to every FV-DNA which is still capable of ensuring all functions of the FV vector which are necessary for the insertion of the foreign DNA and the expression thereof.

The expression "sequence which permits the propagation in bacteria" used herein relates to sequences containing an "ori" for the replication in bacteria, a marker gene and/or a resistance gene. For example, such a sequence may originate from pAT153 (Twiggs and Sherratt, 1980, Nature 283, p. 216).

The inventors found out that two kinds of genetic modification or vector types are advantageous:

- 1) self-replicating, replication-competent vectors in which the insertion of heterologous genes do not impair the replication capacity and which may replicate in the therapeutic host. No packaging cell line or a helper virus is necessary for the self-replicating vectors;

- 2) replication-defective vectors in which e.g. the entire structural genes (gag, env, pol) were replaced by the foreign DNA. The virions form in what is called packaging cell lines which provide all deleted functions in trans (gag, env, pol). These vectors contain the cis sequences necessary for packaging the genomes and expressing the foreign DNA.

Spumaretroviruses/foamy viruses are generally considered nonpathogenic, even though they may trigger diseases under certain experimental (artificial) conditions (transgenic mice). The "apparent nonpathogenicity" of foamy viruses is one of their major advantages as a retroviral vector. However, preferably the strong Bel 1 transactivator should at least be inactivated functionally, preferably be deleted completely, in all of the self-replicating FeFV vectors.

The foreign DNA which can be inserted in the vectors according to the invention is not subject to a restriction and, in principle, all disease-relevant genes are possible. Examples of foreign DNA (also referred to as heterologous genes or therapeutic genes) are

- immunomodulatory genes for activating or suppressing immune responses in the course of anti-cancer therapies, e.g. interleukin-2, -1, -10, etc., gamma-interferon, tumor necrosis factor, special tumor antigens,
- suicide genes for the specific destruction of the correspondingly infected cells, e.g. HSC thymidine kinase, E. coli cytosine deaminase, polynucleoside phosphorylase,
- functionally intact genes for the substitution of functionally defective genes in the course of the somatic gene therapy,
- expression of trans-dominant negative mutant proteins, which are used in an inhibitory manner within the scope of the "pathogen derived resistance" or "intracellular immunization",
- genes whose protein product can be isolated and purified from transgenic animals in suitable form on a

technical/industrial scale, e.g. factor X of blood coagulation or the like.

According to the invention the retroviral FV vector is a vector in which the genome of the feline foamy virus (FeFV) was used. It may be a vector in which the first DNA sequence is the FV-DNA sequence of the vector FeFV-7. This vector was deposited as plasmid pFeFV-7 on November 23, 1998 under DSM 12514 with DSMZ [German-type collection of microorganisms and cell cultures], Mascherorder Weg 1b, 38124 Braunschweig.

The first DNA sequence of the vector may, however, differ from the DNA sequence of the pFeFV-7 vector and may only be derived therefrom. The FeFV portion of the plasmid can be modified, for example, such that it is changed genetically in accordance with the requirements of the vector concept (self-replicating or replication-defective vector) and that the foreign DNA (also referred to as heterologous gene or therapeutic gene above) is inserted under the transcriptional control of FeFV or a heterologous promoter. For example, the first DNA sequence may also differ from that of the pFeFV-7 vector according to the invention as regards its length, or may have various nucleotide additions, deletions or substitutions, e.g. originate from another field isolate of FeFV as long as the desired properties of the virus, e.g. as regards infectiosity, replication, insertion and expression of the foreign DNA are maintained for the desired purposes according to the invention. A person skilled in the art can determine by means of common methods, e.g. the methods indicated in the below examples or in Winkler et al., J. Virol. 71 (1997), 6727-6741, whether the DNA sequence used for the production of the retroviral FeFV vector still meets the necessary conditions. In addition, general methods known in the special field can be used for the construction of the retroviral vectors according to the invention. These methods comprise e.g. common *in vitro* recombination techniques, synthetic methods and *in vivo* recombination methods.

The desired foreign DNA to be expressed may be inserted at

any site of the FV genome, provided that its expression is ensured by this and the desired properties of the FV (e.g. self-replicating or replication-defective vector) are maintained. In principle, the foreign DNA may be inserted at any site of the FeFV genome. However, it should be such that the FeFV portion is still active in the above-mentioned sense (self-replicating/replication-defective vector). The foreign DNA sequence is preferably inserted between the 5'LTR region and the 3'LTR region. In the case of self-replicating vectors heterologous sequences may be inserted in the Gag, Env and Bel2 genes or in defined portions of the 3'-LTR; in the case of replication-defective vectors, the Gag, Pol, Env and accessory/regulatory genes are preferably replaced by the foreign DNA to be expressed. In order to obtain an efficient expression of the foreign DNA sequence, it is necessary to insert it in the VF vector such that it is present in functionally linked fashion with suitable transcriptional control sequences. Suitable control sequences suitable for the expression in mammals (i.e. promoter and/or enhancer sequences) are known to the person skilled in the art. The promoters known to the person skilled in the art are usable as promoters. They comprise e.g.

- the genetically modified FeFV LTR promoter or the internal promoter,
- constitutively active promoters, e.g. HSV-tk, CMV-IE,
- cellular housekeeping promoters, e.g. beta-actin, GADPH,
- cell-type-specific promoters for the expression in hematopoietic cells, the CNS, the liver, kidney, etc.
- inducible promoters, e.g. promoters dependent on insulin, corticoid, stress, estrogen,
- promoters which can be activated by viruses, e.g. HIV LTR for intracellular immunizations,
- promoters which are activated by established therapeutic agents (tamoxifen, etc.).

In a particularly preferred embodiment the retroviral FV vector also contains a gene which codes for a detectable phenotypic marker. This permits checking a successful transformation of the desired target cell. Suitable marker

genes are e.g.:

- β -galactosidase
- resistance gene against neomycin, hygromycin, zeonin, etc.
- (humanized) green fluorescent protein GFP.

The present invention also relates to a plasmid which contains the FeFV vector according to the invention. The plasmid may be replicated e.g. in *E. coli*, in *E. coli* JM 109 or DH5 α , for example, and be isolated directly therefrom.

Methods of transforming the cells for the production of the FV vector according to the invention (e.g. via CaPO₄ precipitation, liposomes or electrotransformation, etc.) and for the phenotypic selection of transformants are known in the art.

After the replication, the FV vectors according to the invention may be inserted in a cell, a tissue, organ, a patient or an animal by a number of methods. The FeFV (and thus also the pFeFV-7 plasmid derived therefrom) may replicate in cells of human origin. No absolutely essential changes of the plasmid pFeFV and its derivatives are necessary for the replication and application in human cells and humans so that FeFV can also infect these human cells. The above-mentioned methods and the co-culturing using vector-producing cells and direct infection with the retroviral vector are in consideration for the *ex vivo* application. The following are in consideration for the *in vivo* application: as DNA by the gene gun; liposome technique; as a free retrovirus systemically (e.g. intravenously), locally (e.g. directly into a tumor or the lymphatic organ) or in the form of an aerosol for the respiratory tract. In analogy, it is also possible to administer the vector-producing cells.

Thus, the present invention also relates to cells and transgenic animals (i.e. mammals which are transgenic with respect to the DNA sequence inserted by means of the FV

vector according to the invention). Methods for the production of such transgenic animals may be found e.g. in WO 91/08216 or Schenkel, Johannes, 1995, *Transgene Tiere* [Transgenic Animals], Spektrum Akad. Verlag, Heidelberg, Germany.

The present invention also relates to medicaments which contain the above described FV vectors. These medicaments optionally contain in addition a pharmaceutically acceptable carrier. Suitable carriers and the formulation of such medicaments are known to the person skilled in the art. Suitable carriers are e.g. phosphate-buffered common salt solutions, water, emulsions, e.g. oil/water emulsions, wetting agents, sterile solutions, etc. The kind of carrier depends, of course, on how the FV vector according to the invention is to be administered. The suitable dosage is determined by the attending physician and depends on various factors, e.g. the age, sex, weight of the animal or patient, the severity of the disease, the kind of administration, etc.

The FV vector according to the invention may also be used e.g. for the expression of DNA sequences which code for neutralizing epitopes of pathogens, i.e. it may be used for a vaccination against these pathogens. This is of interest above all as regards the infectious diseases, e.g. in cats, for which no suitable vaccines have been available thus far. In this connection, it is to be noted that the expressed epitopes are therapeutic (immunologically) active domains and the vaccination does not lead to e.g. an immunopathogenesis in the case of subsequent virus challenge. For example, the foreign DNA sequence may code for neutralizing epitopes of the feline immunodeficiency virus (FIV). In this case, an FV vector is obtained which permits the efficient vaccination of cats over FIV. Other suitable foreign DNAs are viral Env surface domains or T-cell epitopes of viral structural or non-structural proteins. As stated above already, the use of the subject matters according to the invention is by no means limited to vaccination but represents generally a vehicle for gene therapy.

In order to demonstrate the feasibility of FeFV-based vectors, the epitopes, relevant for virus neutralization, of the FUV isolate used by the inventors were exchanged by the corresponding sequences of an FeFV virus similar to isolate 951 (Flower et al., 1985, Arch. Virol. 83, p. 53). The genetically modified virus is replication-competent and due to the exchange of neutralization-relevant epitopes will no longer be neutralized by sera against the FeFV-FUV isolate. Due to its distinct immunological characteristics this genetically modified, now 951-similar FeFV may possibly then be used in cats (or humans) as a gene-therapeutic vector if a humoral immunity against the original FUV-similar vector is already present. Such a situation may exist if the therapeutic use of the vector must be carried out repeatedly. Thus, the availability of the immunologically distinct FeFV vectors can permit a substantially more flexible use of these recombinant viruses in the therapy. In spite of existing immunity against an FeFV virus isolate therapeutic applications are still possible with the other FeFV serotype, which is an advantage that none of the other retroviral vector systems has.

The FV vector according to the invention is not only suitable for the gene therapy/vaccination of animals, e.g. cats, but also for the corresponding therapies in humans. Due to the fact that (a) with the above described FeFV vector according to the invention cats can be vaccinated against FIV, and (b) the FIV infection of the cat is strongly similar, from a molecular-biological and pathogenetic view, to the HIV infection of humans (see e.g. in this connection Elder et al., Aids Research and Human Retroviruses 14 (1998), 797-801), it may be assumed that the FV vector according to the invention is not only suitable for gene therapy in humans as regards e.g. the introduction of tumor suppressor genes but also useful for carrying out a vaccination against HIV. For a vaccination against the HIV infection primary T cell epitopes and sequences of the HIV structural and non-structural proteins offer themselves, which carry immunorelevant epitopes, on the one hand, and at the same time are conserved

at least moderately. The inventors confirmed that feline and human cells replicate the vector according to the invention, and recombinant vector particles form in both cell types (e.g. feline CRFK cells, human 293T cells).

Finally, the present invention also relates to the use of the FV vector according to the invention for vaccination, preferably against FIV in cats or HIV in humans.

The figures show:

Figure 1: diagram of the organization of the FeFV provirus

Figure 2: nucleic acid sequence and derived amino acid sequence of FeFV (Winkler et al., J. of Virology, Vol. 71, No. 9, Sep. 1997, pp. 6727-6741)

Figure 3: map of the clone pFeFV-7 (length: 14.463 bp)
The portions corresponding to pAT153 and FeFV are characterized. Moreover, the positions of the most important restriction sites are indicated.

The below examples illustrate the invention.

Example 1

Production of an FeFV vector (pFeFV-z)

The production of FeFV isolates, the DNA extraction, the construction of recombinant FeFV DNA clones and the analysis of these clones was made as described in principle in Winkler et al., J. Virol., 71 (1997), 6727,6741. FeFV DNA sequences of the nucleotide positions 5118 to 7431 were carried out by "long-template PCR" using specific primers and the DNA of "Crandell" feline kidney cells (CRFK cells) infected with FeFV. The primers are:

5118: 5'-CCTCATGCTTACGGGAATAATCTGGCTG-3' (forward)
7431: 5'-GAATAGCATACCAGAGCCTACAGGGCTC-3' (reverse)

7163: 5'-CCAATTGGACAAGAGTAGAATCCTATGG-3' (forward)
9057: 5'-TTCTCCAAGGAGCTGCAGCCACTCTGG-3' (reverse)
5775: 5'-TTTGCTCAGTGGGCAAAGGAAAGGAATATACAATTGG-3'
(forward)
10522: 5'-GTTGACACTGATTTATATGGCACAATAATTCCTCTC-3' (reverse)

The resulting amplified DNA was cloned in pCRII vectors (Invitrogen company, Netherlands) by means of common methods. Correct recombinant DNA clones were excised using NdeI (in FeFV) and Ec1136II (in the pCRII vector) and the resulting fragment of about 2.3 kpb was inserted in the FeFV-DNA clone 7 (cleaved by ClaI, thereafter provided with blunt ends by means of Klenow polymerase and finally excised using NdeI). Clone 7 contains FeFC-DNA sequences from nucleotide positions 17 to 5811 (Winkler *et al.*, 1997). By this cloning FeFV clones were obtained which extended from the nucleotide position 17 to 7431 (clones 24 and 28).

At the same time, the FeFV insertion was subcloned from the recombinant clones 4,6 and 8, which contained FeFV-DNA sequences from nucleotide positions 8636 to the 3'-end of the 3'LTR (Winkler *et al.*, 1977), into the vector pBluescript KS (Stratagene company, Heidelberg, Germany), the common flanking enzyme restriction sites of the polylinker having been used. In the resulting clones 5,7 and 15 ClaI and ApaI restriction sites from the PCR primers and the polylinker site of the vector follow the 3' end of the genome. FeFV-DNA sequences from nucleotide positions 7163 to 9057 were amplified by "long-template PCR" using the above-mentioned primers and cloned into the plasmid pCRII as described above, the clone V being obtained.

For the production of a clone which contains the complete proviral DNA, the following cloned, above described DNA fragments were linked with one another in a triple ligation: The ApaI (vector restriction site)/Pml I-fragment of clones 24/28 + the PmlI/PstI fragment of clone V + the PstI/ApaI fragment of clones 5, 7 and 15.

The resulting DNA clones were genetically stable and contained FeFV DNA from nucleotide positions 17 to 11700 as an insertion in the pBluescript vector. The FeFV insertion between the EagI and ClaI sites which flank the FeFV DNA, were then subcloned into the correspondingly excised vector pAT153 (= "high copy-number" deletion mutant of pBR322; Twiggs and Sherratt, 1980, Nature 283, page 216).

Various independently obtained clones were analyzed after the transfection in FeFV-permissive eukaryotic CRFK cells. However, little infectiosity showed. Clone 13 showed a moderate viral infectiosity and was therefore used for the complete restauration of the infectiosity. FeFV-DNA sequences from nucleotide positions 5775 to 10522 was amplified by means of long-template PCR using the above-mentioned primers and excised directly with BstZ17I (nucleotide position 5980) and BsaI (nucleotide position 10137). The resulting DNA fragment having a length of about 4.2 kbp was used for exchanging the corresponding sequences of clone 13 (with complete length). After the transfection in CRFK cells it showed that the resulting FeFV clone pFeFV-7 was fully infectious and genetically stable. The resulting recombinant FeFV virus could not be distinguished from non-cloned viruses obtained from cell cultures. pFeFV-7 was deposited on November 23, 1998 under number DSM 12514 with DSMZ, Mascheroder Weg 2, Braunschweig, Germany.

Example 2

Production of a neutralization-resistant variant of pFeFV-7 expressing heterologous sequences of the FeFV serotype 951 in feline cells

In order to demonstrate the feasibility of FeFV-based vectors, the epitopes of the above described FeFV-7 isolate which are relevant for the virus neutralization were exchanged by the corresponding sequences of an FeFV virus similar to isolate 951 (Flower et al., 1985, Arch. Virol. 83, 53).

The following steps were taken:

The Env DNA sequences of the FeFV isolate 951 were amplified from DNA of FeFV-951-infected CRFK cells with the forward primer 5-GACATACCTGAAGATATTC-3' (position 6418) and the reverse primer 5'-CGACTTGTACCAGGCCTATTCCTGG-3' (position 9901) by means of PCR. The amplificate was digested by means of the restriction endonuclease KpnI and the 3.5 kB long DNA fragment was isolated. The above-described FeFV vector pFeFV-7 was digested simultaneously with Kpn and the DNA fragment corresponding to the vector backbone was isolated. Both DNAs were ligated with each other and the recombinants were identified, in which the FeFV-951 env DNA sequences had been inserted in proper orientation in pFeFV-7. The resulting DNA clone pFEFV-7/951 is genetically stable. Following transfection in CRFK cells the protein expression pattern of this clone cannot be distinguished from the wild-type clone pFeFV-7. Plasmid pFeFV-7/951 induced the synthesis of infectious FeFV particles.

The genetically modified hybrid pFeFV-7/951 is replication-competent and is no longer neutralized by sera against the FeFV isolate due to the exchange of neutralization-relevant epitopes. This was shown in neutralization studies: Sera which are directed against the FEFV isolate, neutralize descendants of the plasmid FeFV-7 but not viruses of the pFEFV-7/951 clone. Sera against the FeFV isolate 951 neutralize correspondingly viruses of the pFeFV-7/951 clone but not of plasmid pFeFV-7.

In this example, a chimeric hybrid vector was thus constructed which expresses in efficient and stable manner heterologous protein sequences. In addition, a vector pair (plasmids pFeFV-7 and pFeFV-7/951) was constructed for the first time which induces no cross-neutralizing antibodies.

In a therapeutic application of both vectors, thus a serotype (e.g. FeFV-7) may first be used for the transfer of the therapeutic gene. If a patient establishes a neutralizing seroreactivity against this vector serotype, the other serotype (in this case pFeFV-7/951) may be used, against

which no neutralizing antibodies exist. Therapeutic applications would still be possible with the other serotype in spite of an existing immunity against a virus isolate. This is an advantage which no other retroviral vector system has.

Article
34

12. Juni 2001

09868502 09868502
097868502

JC18 Rec'd PCT/PTO 1 8 JUN 2001

K 2978

Amended Claims

1. A retroviral vector for introducing a desired, expressible DNA into a mammalian cell, wherein the retroviral vector comprises the following sequences: A first DNA sequence corresponding to the reverse transcript of at least part of a feline foamy virus (FeFV), and a second DNA sequence permitting the propagation in bacteria.
2. The retroviral vector according to claim 1, wherein the vector further comprises a desired expressible foreign DNA.
3. The retroviral vector according to claim 1 or 2, wherein the first DNA sequence comprises the reverse transcript of the entire foamy virus.
4. The retroviral vector according to any one of claims 1 to 3, wherein the first DNA sequence comprises both 5'LTR and 3'LTR of a foamy virus.
5. The retroviral vector according to any one of claims 1 to 4, wherein the second DNA sequence is inserted in the 5'LTR region and/or 3'LTR region of the foamy virus.
6. The retroviral vector according to any one of claims 1 to 5, which additionally contains a gene coding for an identifiable phenotypic marker.
7. The retroviral vector according to any one of claims 1 to 6, wherein the foreign DNA sequence codes for neutralizing epitopes of the feline immunodeficiency virus (FIV) or the human immunodeficiency virus (HIV).

- 5 8. The retroviral vector according to any one of claims 1 to 7, wherein the first DNA sequence is that of the plasmid pFeFV-7 deposited with the DSMZ (German Type Collection of Microorganisms and Cell Cultures) on November 23, 1998 (DSM 12514).
9. A plasmid containing the retroviral vector according to any one of claims 1 to 8.
- 10 10. A cell containing the plasmid according to claim 9.
11. The cell according to claim 10, which is an animal cell.
12. The cell according to claim 11, which is a mammalian cell.
- 15 13. A transgenic animal containing the retroviral vector according to any one of claims 1 to 8 or the plasmid according to claim 9.
- 20 14. Use of the vector according to any one of claims 1 to 8 or the plasmid according to claim 9 for the production of a medicament for vaccination.
- 25 15. Use of the vector according to claim 7 for the production of a medicament for the vaccination of cats against FIV or human beings against HIV.
- 30 16. Use of the vector according to any one of claims 1 to 8 or the plasmid according to claim 9 for the production of a medicament for the gene-therapeutic treatment of patients.

Abstract of the Disclosure

The invention relates to retroviral vectors based on feline foamy viruses (FeFV) for introducing a desired, expressible DNA into a mammalian cell. An example of the vector according to the invention is a vector which permits the expression of neutralizing epitopes of the feline immunodeficiency virus (FIV) in cats or of the human immunodeficiency virus (HIV) in humans and thus enables an effective vaccination.

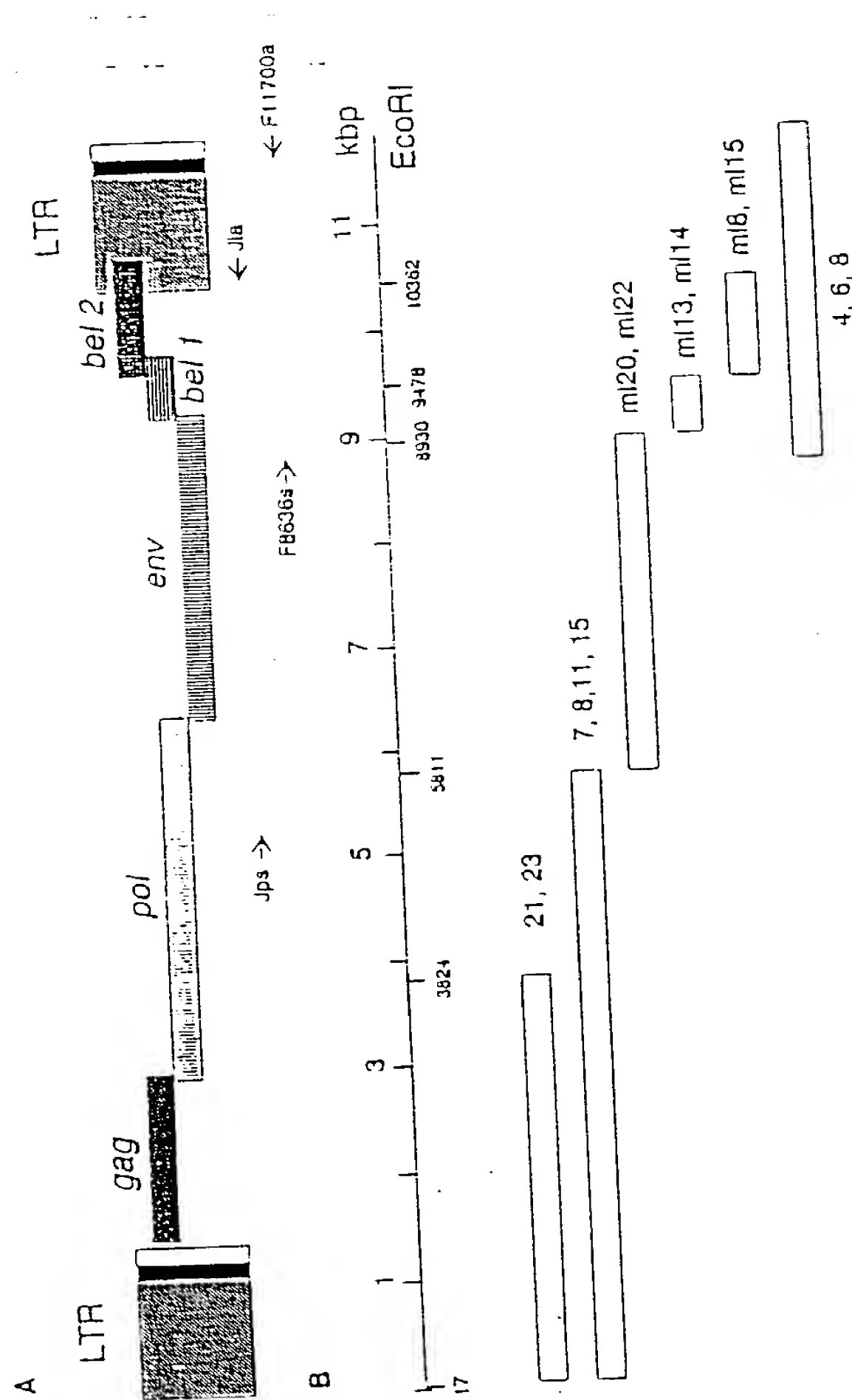


Fig. 1

2/7

PBS
 1356 TGGCSCCAACGTGGGGCTCGATCGAGTGAATTTAAATTAAGCTGAGSAGAATAATCCCTAGGGACCTTACCTTACTGAGGAGGATGGCTCGAGATT
 gag M A R E E E

1456 AAATCCTCTCCAAATTACAGCACTGTATTAATTAATGGCTTACACCTAATCCAGGACCTGAGATATTATTGCTGTGAGATTACAGGAGGACCTTGG
 N P L Q L Q Q L Y I N N G L Q P N P G H G D I I A V R P T G G P W

1556 GGTCCAGGTGATAGATGGGCTAGAGTGAACAATACBATTACAGATAACACAGGACCAACCTTACAGGTTCCTGCTATGATTTTGGAACTGGGATTAAT
 G P G D R W A R V T I R L Q D N T G Q P L Q V P G Y D L E P G I I N

1656 ATTTGAGAGAGGATATCTTGATAGCAGGGCCATATAATTTAATAAGAACTGCTTTTGGACTTAGAGCCTGCGCAGGAGACCTGAAAGGCATGGTCCCTT
 L R E D I L I A G P Y N L I R T A F L D L E P A R G P E R H G P P

1756 TGGAGATGGCAGATTACAGCCTGAGATGGTTTATCTGAGCAATTTCACTTATCTCTGATGAAGAAATACAGCAGAAAGTAGGAACCTTGGTCTGCT
 G D G R L Q P G D S L S E G P Q P I T D E E I Q A E V G T I G A A

1856 AGAAATGAGATAAGATTATACAGAAAGCTTACAGAGATTACAGCTGAGGTGTGGGTAGACCTATACAGGAGCAGTTTACACCAACCAACCTGTA
 R N E I R L L R E A L Q R L Q A G G V G R P I P G A V L Q P Q P V I

1956 TAGGGCCCGTAATACCAATTAATCATCTTACGTCGGTATTGGCAATCTCCACCAATTCACAGATGTGCGCTATGGCTTGGAGATCTACAGCCGC
 G P V I P I N H L R S V I G N T P P N P R D V A L W L G R S T A A

2056 TATTGAAGGAGTATTTCCCACTAGTGAACCTAGTCACTCTATGAGGGTAGTTAATGCTTAGTAGCATCTCTATCCGCGCTAACCTTGAAGTGAATGAG
 I E G V P P I V D Q V T R M R V V N A L V A S H P G L T L T E V S

2156 GCGGGAGCTGGAATGCTGCCATATCGCTTATGAGGAAAGCTCACGCTGCTGAGCTCAGCTGAATTGGCAGGAGTATTAAGTGAATTAATAAA
 A G S W N A A I E A L W R K A H G A A A Q H E L A G V L S D I N K K

2256 AGGAAGGCATACCAACTGCACTGACCTAGGAATGCAATTAACAGATGGAAGCTGGTCTTAGTATGGGGAATTAACAGGACTCTTTACAGGACACAGC
 E G I Q T A F N L G M Q F T D G N W S L V W G I I R T L L P G Q A

2356 CCTAGTAACCAATGCTCAGTCACTTACCTAATGAGAGATGATATACACAGCAGAAATTTCCGAGGGTCAATTAACATCTATACCTATGCTG
 L V T M A Q S Q F D L M G D D I Q R A E Y P P R V I N N D Y T M L

2456 GGTCTCAATATACATGGGCAAGTATTAGACCTCGGCTCCTAACACAGCCTCTACAACTCGACCCGCGAACCCTGGAGCATCTCAACAGGTCACTAA
 G L N I H G Q S I R P R V Q T Q P L Q T R P R N P G R S Q Q G Q L N

2556 ATCAGCCGAGACTCCCAAAATAGAGCTAACCTATCTTACAGCCCTGAGCAACAGCAGCACTCTGATGTTCCGCAACAGAGAGATCAGAGAGGACC
 Q P R P Q N R A N Q S Y R P P R Q Q Q Q H S D V P E Q R D Q R G P

2656 GTCCCAACCTCTCTTGGAGTGGAGGAGCTATAATTTAGAAGAAATCCGACGAGCCTGAGCCCTACCGGCAAGGACCTACCGGACCAACCCCTAC
 E Q P P R G S G G G T N P R R N P Q Q P Q R Y G Q G P P G P N P Y

2756 CGACCAATCGAGACCGCGCTAATCTTACAGCAGGAGCTCCTACCAACCGAGGCGCTGATCAAGGACCTCGGCAAGGAGCAATCCAGAGGAGGAG
 R R P G D G G N P Q Q Q Q P P P N R G P D Q G P R P G G N P R G G G

2856 GAAGAGGTCAAGGTCCAGAAATCGAGGAGGAGCGCTGCGGCACTACCTACGTAAGAGCTCTGAAAACCAAACTAAAAATGATCTGCTGAAGCGT
 R G Q G P R N G G G S A A A V H T V K A S E N E T K N G S A E A V
 Pol M D L L X P L

2956 TGACCTGGAAAGAAAGGGGTAAAGATTAAAGTTACTGGGACTCCCAAGCCGATATTACCTGTCTTCCAAAGGACTTCTTAAAGGAGAAAGACCTGT
 T V E R K G V K I K G Y W D S Q A D I T C V P K D L L Q G E E P V
 D G G K K G G K D

3056 TAGGCAGCAAAATGTGACTACTTATCATGAAAGCAGGAGGAGATGTAATTTATGAATTTAAAAATGACGCTAGAGAAATTAATCAAGATTAATA
 R Q Q N V T T I E G T Q E G D V Y Y V N L K I D G R R I N T E V I

3156 GGGACAACTTTGGACTATGCTATTAATCTCTGAGATCTACCTGCAATTTGAGAAACCTTAGAATTAATTAACCTGATTAAGAGGAGCAGC
 G T T L D Y A I I T P G D V P W I L K K P L E L T I K L D L E E Q Q

3256 AAGGGACTTTACTTAACAAATCTCTTTCTTAAAAAAGGGAAGAGAATTAACAATTAATTTGAGAAATATAGTGCCTTATGGCAAGTTGGGAGAA
 G T L L N N S I L S K K G K E E L K Q L F E K Y S A L W Q E W E N

3356 TCAGGTGGGTCTAGAGAAATTAAGGCTCCTAATAATAGCACTGCTTACGTAAGAACTTACCTTGAAGAACAGTATCTATTAATCCAAAGGCAAACTT
 Q V G H R R I R P H K I A T G T V K P T P Q K Q Y H I N P K A K P

3456 GATATTGAGATTGTGATTAATGATTTACAAACAGGCGGTACTAATTCAAAGGAAAGTACTATGAACACTCTCTGTCTACCTGCTACCGAGCAATG
 D I Q I V I N D L L K Q G V L I Q K E S T M N T P V Y P V P K P N G

3556 GTCCCTGCAAGATGGTACTGCACTACAGGCACTAAATTAAGTCACTCTTGAATGCTGTACAAATCAACACTCGAAGGAATTTAGGAGTCTTTT
 R W R M V L D Y R A V N K V T P L I A V Q N Q H E Y G I L G S L P

3656 TAAAGGTAGATTAATACTCAATTAATTAATGATGCTTCTGGGCTCAGCCCTAGCTCCAGAGGATTAATGATTAATCTGATTAATCTTGGCAGGA
 K E R Y K T T I D L S N G P W A E P I V P E D Y N I T A P T W Q G

Fig. 2

3756 AAACATATTTGTTGGAGCTGTTTACACACAGGTTTCTTAAACAGGCGCTGGGTTGTTTACTGGAGATGTTGTAGATCTTCTACAGGGGAATCCCAACGTGG
K Q Y C W T V L P Q G F L N S P G L S T G D V V D L L Q G I P N V Z

3856 AAGTCTATGTGGACGATGTATATATTAGTCAAGATTCTGAAAAGAACCTTTGGAAATATCTGGATATTTTGTTTTAAAGATTAAGAAGCCAGGATATAT
V T V D D V Y I S H D S E K S E L E Y L D I L P N R L K E A G Y I

3956 AATATCTCTTAAAAAATCCAAATATTGCCAATCTATTTGTTGGATTTTCTTGGTTTTCAGATTACTAATGAAGGCCGGGGCTGACAGATCTTTTAAAGAA
I S L K K S N I A N S I V D F L G P Q I T N E G R G L T D T F K E

4056 AAATGGGAAAGATTTACTGCCCCCTACCACTCTTAAACAAATTCGAAAGCCTACTAGGTCTTTTAAATTTTGGCGAAATTTTACTCTCTCACTTACTGAAT
R L S N I T A P T T L K Q L Q S I L S L L N F A R N F I P D S T S L

4156 TAATTCTCTCTTATATGCACTTGAAGCAAAAGTCTACCAAGAAATAGGTTCTCTGGCAATAGAACCTTCAACCACTCTCGAAACTTTAACTTACTTAAACT
I A P L Y A L I P K S T K N Y V P W Q I E E S T T L E T L I T K L

4256 AAACGGGGCGAATATTTACAGGAAGAAAGGAGATAAACCTTCATCTGAAAGTCTATGCTAGTTATACAAACGGATATATAGGTATTATATATGA
N G A E Y L C G R K G D X T L I M K V N A E Y T T G Y I R Y Y N E

4356 GGGGAAAGAACCCATATCTTATGTAGTATAGTGTTCGCAAACTCAATTGAAATTCCTGAACTAGAGAAATTECTGACCACTCTGCAACAGGGTGC
G E K K F I S Y V S I V P E K T E L K P T E L E K L L T T V H K G L

4456 TTTTAAAGGCTTGGAGCTTCTCATGEGGGCAAACTTCACTGTTTCTTCTGCTTCTATCCATGCAAAATATTCAAAAAACCCCTGAGCTGCTTAAAA
L K A L D L S M J Q N I H V Y S P I V E M Q N I Q K T P Q T A K K

4556 GGCTTTGGCCTCTCATGCTTGGAGTTGGCTTTCTTATTTGGAAGATCCAGAAATTAGGTTCTTTTATGATCCACAGAGCCCTGCTCTTAAAGATTTGGCT
A L A E R W L S W L S Y L E D P R I R E P Y D P Q M P A L K D L P

4656 GCTGTAGACACCCGAAAGATTAATAAAAAACCTCTACCAATTTTCAACATATATTTTCACTGATGCTTCTGCTATCACTCTCTTACTTACAGGAGGAC
A V D T G K D N K X E P S N F Q H I P Y T D G S A I T S P T K E G H

4756 ATTTAAACCTTGGAAATGGGAATAGTTTATTTTATTAACAAAGATGGAATTTACAAAGCAACAGGAATGCTCTCTTACTTTGGGGAATCATACAGCACA
L N A G M G I V Y P I N K D G N L Q K Q Q E W S I S L G N E T A Q

4856 ATTTGCGAATAAGCTCTTTTGGAGTTTGGCTTAAAAATGTTTGGCTTTGGGAGGAACATTTCTTGTGTTTACTGACAGCAATTAATGTTGCAAAAGCA
F A E I A A F E F A L K K C L P L G G N I L V V T D S N Y V A K A

4956 TATAATGAGGAATCTGATGTTTGGGCTCTAATGGCTTTGGAATAACAGGAAGAACCTTTGAACATATTAGTAAATGGAATCGGTTGCTGACCTTA
Y N E E L D V W A E N G P V N Y R K K P L K H I S K W K S V A D L K

5056 AAAGATTAAGGCCAGATGTTGTTGTGACCCCTGAGCCAGGTCACAAAAAATCTGACTCATCTCTCATGCTTACCGGAATTAATCTGGCTGATCACTGGC
R L R P D V V V T H E P G H Q K L D S S P H A Y G N N L A D Q L A

5156 CACGCAAGCCAGTTTAAAGTACATTTGACTAAAAATCCCAAGCTGACATTGAGCAAAATTAAGGCAATTCAGGCACTGTCAAAATTAATGAAGATTACTT
T Q A S P K V H M T K N P K L D I E Q I K A I Q A C Q N N E R L P

5256 GTTGGTATCTAAAAACAATATACCTATGAGTTGCAAAATTAATGATGAGTTTAAAGAAAGACGGTTGGAGGGAAATTCCTCTCTTCCGAGAACCGT
V G Y P K Q Y T Y E L Q N N K C M V L R K D G W R E I P P S R E R Y

5356 ATAACTTATTAAGAAGCACCTTACCTTACTGCTGCGGCGGAGAGCCCTGTTTATTAATAATACAGAAATTTATTGGTGGCCAAAAATGAAGAAAGA
K L I K E A E N I S H A G R E A V L L K I Q E N Y W W P K M K K D

5456 TATATCATCTTTCTTCTACATGTAATGTAATGTAAGATGGTAAATCTTTGAATTGAAGCTTATTAGCCCTCAAGCAATTTGTAACCCACCAACCTT
I E S P L S T C N V C K M V N P L N L K P I S P Q A I V H P T K P

5556 TTTGATAAATTTTATATGGATTACATTGGGCCATTGCGACCTTCAAGGTTATGTCATGTTTATGTTGGTGGTATGATGCTGCTGATTTACTTGGT
P D K F Y M D Y I G P L P P S E G Y V H V L V V V D A A T G P T W L

5656 TGTACCCCACTAGGCTTAAACCTCGAAGGCCCAATTAAGTTCTTATCTCTCACTGGAAGCAATTCGAAGGTTCTGCTCTGATCAAGGATC
Y P T K A Q T S K A T I K V L N E L T G L A I P K V L H S D Q G S

5756 AGCAATTACTTCTGAAGAATTTGCTCAGTGGGCAAGGAAAGGAATATACAAATGGAATTCAGTACTCTTACCAACCTCTCAAGTATGTTGGAAAGTGGAA
A P T S E E P A Q W A K E R N I Q L E F S T P Y H P Q S S G K V E

5856 AGGAAAAACAGTGAATTAAGAAATTTTAACTAAGCTCTTGGTTGGGAGGCTTTTAAAGTGGTATTAACCTTATATCCAGTGTGCACTTGTCTTAAATA
R K N S E I K X L L T K L L V G R P L K N Y N L I S S V Q L A L N N

5956 ACACTCATGTTGTGAGCACAAGTATCTCTCTCATCACTAATGTTTGGAAATGATTTGTAATTTACCAATTTGCTAATTAAGGATACCTTGGACTGGACAG
T E V V E T K Y T P H Q L M F S I D C N L P P A N K D T L D N T R

6056 AGAAGAAAGAACTTGGCTCTCTTGGCAGGAAATCTGGAATCTTTACACACCCCTTACCAACCCCTGCTCTGCTGGTGGTCACTTACTTGGCAGCTG
E E E L A L L Q E I R E S L Q H P V Q P P T C S G W I P Y V G Q L

6156 GTCCAGGAGAGGCTGTACAGGCCCTTCACTAAGGCTTAAAGTGGAGGAGGCTTCAAGGCTTTGGAATTTGTAATCTTGAAGTGTGATTTATAGTGG

2. PPT

Fig. 2 (cont'd)

V Q E R V Y R P S Q L L R P K W R K P T K V L S I L N P R T V I I V D

6256 ACCATCTAGGCGACGGAAATCTGTGAGTATTGACIATTTAAAACSTACAGCOCACCGGCATAATGGGAACAAGAACATTTGATGACCTTGAGGAATGGA
H L G Q R K S V B I D N L K P T A H Q H N G T R T C D D P E G M D
Enr M E Q Z H V M T L X Z W M

6356 TGGAAATGGAATGCTCACAAAACACTACGAAACTTCAGTCGACTCATCTGTGAGTTCATGTTGACATACCTGAGGATATTCTTTTAGTACCAGAGAGGT
G M E C S Q T T T E T S V D S S
E W N A H K O L Q K L Q S T H P E L R V D I P S D I P L V P E K V

6456 ACCTTTGAAAAATGAGGATGCGAATAGAGTTTATCTTTGTCTACTTCTACTAGATAATGTTTTGGATACTATTCTTCTTCTATCTTTTCAATA
P L K M R M R Z R C Y T L C A T S T R I M F W I L P F L L C F B I

6556 GTTACCTTGAGTACAAATTACAGTATCTTACATATCATGGAAGAAGCAATACACATCTCTGCCCGAGTCTTAAGCTGGCAGGTGACTAATTCACATG
V T L S T I I S I L R Y Q W K E A I T H P G P V L S W Q V T N S H V

6656 TAACCATGGGAGGAAATACCTTCTCTCTCTCCAGACCGACCTGATACACATACCAAACTTCCCCTAGAGGTTAACCTCTCGGGATCCCCACAGG
T M G C N T E S S S R R R D I Q Y H K L P V E V N I E G I P Q G

6756 TCTTTTCTTCCACCTCAGCRAAACTTATTTTACAAAGAAAGAACTTAAAGTCTTTTCTCAGTGAATCTTATTTACTCTGATACTATTACTCAGGT
L P F A P Q P K P I F H K E R T L G L S Q V I L I D I D T I T Q G

6856 CATATTAAACACAGAAAGCATATTAGTCTCACAATTAAATGAAGAGATGGAGCAATTACAAAGACAGTATTACCTTTTACTTACCATCAGGACC
B I K Q Q K A Y L V S T I N S E M E Q L Q K T V L P P D L P I K D P

6956 CTCTRACTCAAAGCAATACATRGAAAAGGTGCTTTCAAAAATATGGACATTTTATGTATAGCTTTTAAATSGAAATAAAGTTTGGCTTCACAGA
L T Q K E Y I E K R C F Q K Y G H C Y V I A F N G N K V W P S Q D

7056 TTAAATACAGATCAATGTCCATCTCTCTCTCTTGGAAATTAAGTATAGGAACCACTATATGGAAGTATTATATACCTTGCCTTAA
L I Q D Q C P L P P R F G N N L X Y R N E T I W K Y Y I P L P P K

7156 GTATCTCTCAATTGGACAGAGTATAGATCTTATGGAATATTAGGRAGGCGAGCTTAAAGTCTCTGATGAATTTAGACAAATSCACACATSGAATAT
V S S N W T R Y E S Y G N I R I G S F K V P D E F R Q N A T E G I F

7256 TTTTCTGATGCACTATATAGTAAATTTGATCTCAGCTGATCTACCTTCTCTGATACAAATCTTTTCTCAGGCAATATTAACAAAGGTACTTATGA
C S D A L Y S N W Y P R D L P S S V Q Q S F A Q A Y I T K V L M X

7356 AAGGAAAAAGCAACTTACTTTACAGATATAGCTTTTCAAAGCAACTGAGCCTTGTAGGCTCTGTATGCTATTTCAGACCTTTAACCTATATGATATC
R K K Q P T L R D I A F P K E L S P V G S G M L F R P I N P Y D I

7456 TGTAATATGCCAGAGCAGTATTAATATTAATAAAACATATATCTTTCTCAGTATGGGAGGAGATTGTGGATATTACCAACCAATCTTACTCTTC
C N M P Z A V L L L N K T Y Y T P S L W E G D C G Y Y Q H N L T L E

7556 ATCCCTCATGTAAAGAACTTCAATAGAACTGACAGAGACCTTCATATGCTTGCAGATTTTGGAGAAACAGATATGACTCTGAGTCACTGCAATGCTATA
P A C K N P N R T R Q D H P Y A C R P W R N K Y D S E E V Q C Y N

7656 TAATGATATCTGTATATATAGACCTTTGTATATGGAAGTGAAGAACTGAGGATTTGGGGATGGCTGGCATATCTGACTCTTTTCTATCCCCATCTGT
Y D M C Y Y R P L Y D G T E N T E D W G W L A Y T D E F P S P I C

7756 ATTGAAGAAAAGCAATCTGGAAGAAAATTTATCTCTGTCTCTGTATTAGCAGAAATGTGTAAATCAAGCCATGGAATATGSTATAGATGAAGTATTAT
I S S K R I W K K Y Y T L S E V L A E C V N Q A M E Y G I D E V L S

7856 CCAACTAGATCTGATTTTGGGAATCTGACTCATCATCAGCAGATSAGGCTTCTATTCCGGTTAAATTTCACTTGGCTTAGATATGAGAAACAAAA
K L D L I F G N L T H Q S A D E A F I P V N N F T N P R Y E K Q N

7956 TAACAAACAAAAACTTCTTTGTGAAGAAAGAAAGGTAGAGACAAAGAGGTCCGTAAATACCGAAACCTAAGAGGATACAGAGGGCAGGCTTAGGC
K Q Q K T S C E R K K G R R Q R R S V S T S N L R R I Q E A G L G

8056 CTGGCCATGCAATCTACTGTGGCTAAGATCTGTACCTGAATGATCAAAATTTAGCCAAGGGAGTACATTTGCTTAGAGATCATGTTGTCACTCTAA
L A Y A I T T V A K I S D L N D Q K L A K G V E L L R D H V V T L M

8156 TGGAGCCCAATTTGGATGATATTGTGTCTCTGAGAGGGGAATACAAATAGAACATATACATAATCACTTACCTCTTTGAATTTGCTTACTTTGAAAA
E A N L D D I V S L G E G I Q I E H I H N E L T S L K L L T L E N

8256 TAGAATTAAGTGGAGGTTTATTAACCAATTCATGCAATCAAGAGAAATTAAGTGTTCAGATTAATATGAAGTAAATAGGAAAATCTGAGGTGCAAT
R I D W R P I N D E W I Q E E L G V S D N I N K V I R K T A R C I

8356 CTTTACATGTCAACCAACCTGGAATCTAAATCTTCTCTCTGAGGAAATATTTATATATAGATATCATCTTCTACCACTTATATACAGGA
P Y N V K Q T R N L N T S T A W E I Y L Y Y E I I I P T T I Y T Q N

8456 ATTGAATTAAGAAATCTAGGTCACTTTTGAAGAAATGAGGATATTTATCAAGGTGTGATTCACCAACCAATTTGAAGTACTAAACCGGAATGTGG
W N I K N L G H L V R Y A G Y L S K V W I Q Q P P E V L N Q S C G

8556 AACAAATATATATTTACATGCGAAGAACTTTGACCAAGACTATATATATGTGAAGAAGTAATGGAACCTTCTCTCTGTAATGGAAGTCTGCTCA

Fig. 2 (cont'd)

T N I Y L H M E E C V D Q D Y I I C E E V M E L P P C G N G T G S

3636 GACTGCCAGTGCCTAACCAACGCTTACGATGAATACCTTGGAAATGAACCCCTAAAGATGGGAGTTATTTGGTTTATCAAGTACTACAGACTGTG
D C P V L T K P L T D E Y L E I E P L K N G S Y L V L S S T T D C G

8756 GCATACAGCTTACCTGCTTGTGGTTATAACGGTGAATGACACATCAGCTGTTTTGATAAAGAGTTTAAAGGCCACTTAAACAGGAACATAAAGTAAC
I P A Y V P V V L T V N D T I S C F D K E Y K R P L K Q E L K V T

8856 AAAATATGCAACCTTCCCTTCTCAATTAAGACTAAGAGTTCTCTGTTAAACAGGCTTGATTCGAAAAATAAAGGAATTCAATAGAAATTACAGGAGC
K Y A P S V P Q L S L R V P R L T E L I A X I X G I Q I E T T S S

8956 TGGGAACCTATAAAGAGCAGCTGCGAAGGGCCAGGCGAGCTTCAACCTGAGCTTCCAGGAGGAGACTATCCAGAGTGGCTGAGCTCCTTGGAG
W E T I K E Q V A R A K A E L L R L D L H E G D Y P E W L Q L L G E

9056 AAGCACTAAGACCTTGGGCTACATTTGCACTTCTGTTCTGGAATAGGTAATTTATAAAGGACACTGCTGAGGAGCAATTTTGAAGCTGCTTTAG
A T X D V W P T I S N F V S G I G N F I K D T A G G I P S T A P S

9156 TTTTCTGGGATATGTAACCTGTACTTTTGGGATTGTGTATAATATTTTGCATAATTTTAAATTAATAAATCATAGGATGGCTTCAANTACCCGGAAG
P L G Y V K P V L L G P V E E F C I I L I K I I G N L Q N T R K
bel 1 M A S K Y P E E

9256 AAGGACCTAATCTGAGGGGGTTGAAGAAGATTTAACCTCCCTTCTGCTGTTTGAAGCTTACCTCAGCTAATAAAGAGAGCTTTGATTTCTTT
G P I T E G V Z E D F N S H S T S G L D L T S G N K E E P L I S L
K D Q

9356 AGCCCTATTTGCTATGCATACCTGTAATTTGTTTGGATTAAGGGATGCTTTTGTAAAAATATATCTCTTGGAGGGAGCAAGTGTATATAT
A L L S M E T S K I V V W I R D H F P V K I L S P G G K Q K L Y Y

9456 ATATGCAACCTATGTCTAAGGAATTCCTGAAGTGGATACATCTCTCAATACTAATATTATCTATATAGAAAGGACCTACTAGAGCTGGCACC
I C N Q C H K G I P E S G Y I T L N T K Y Y L Y E K G P T E T S T K

9556 AAGGTCTAATCTTATGAGAAGGCATGTGCAAAATTCCTTCTGTTCTGGAAGCTGGAAGAAGATCCGAAACACCTAAGACGATCTACTCTCTCTCTC
G L T L M R R H V Q N S P C P L M S R K E S G T P K T D P T R P A
bel 2 ORF T V G -K -M -P E H P R R I L L V L Q

9656 AACCTCTAATAGCTATGCTAAGCACTATCAGAAGCAGATGTTCCGGGCGCACTCTCTCAATTCCTGAGTCCCTATATAATGGCTTGGGCAACCT
T S Y S L C R S D Y Q E A C C S R P T P S Y S E S V C N G L G Q P
H L I A Y A E A T I K K Q D V P G P L L P I L S P Y V M A W D N P

9756 TCAGAACCTGCTCACACTCTCTGTAATCTGGGGAATCATGGAAGAAGTATCTTTATCTCTGTTTGAAGGATGTGTTGGGAGAGGGATTTGACTATG
S E R G H T S G E S G G I M S E V S P I S W L E G L W G E G P D Y A
Q N V V T R L V N L G E S W K K Y L L S P G W K D C G E R D L T M

9856 CTAACAGAGAATTTGTTGGTACCAGGAATAGGCTGGTACCAATCTCCGCTACACTTACTAATAACCTATGTGTAAATGTGTAAATGGGCGATGTATTACAG
M
L T R E L L V P G I G L V Q I A A T L T K T Y V L M C N G R C I T G

9956 GTTCTAGAACCTACCCAGATTTGTGATCTTTGTTCTGTAAGTTGTATGCTGGAACAAAAATATAAGACCTTAGAGAGTGTAACTAGAGGAATGGTG
S R T D P D C D P L P C K L L C W K Q N I Q D P R E C N L E E W C

10056 CCTGTATAGTCTTGATCTGAAATGATCTCTTGGGATCCAAAAATGATTGTGCTAGACATAGGAATCTTTACCTTATGTATGAGACCTTTCTC
L Y S L D P E H D P L N D P K M I V R R H R N L L P Y C M R P P L

10156 ATTTGGATGAATATATTTCTCAATCTCTTACAGCAATGTATTATGATGAAGCTTTGAATATGCTTTGAGAGCACAAGCTGATGATCTAAGTG
I W M N Y I S E N P L T Q Q C I M M K T L M M L W R A Q A D D P S D
PPT U3

10256 ATGTTGCTTCCCTGTATCCAGAGTCAAGTTTTAAGGCATCTCATTTTACATATTTGGAAGTGCCTCTGGGAACAGTCAAGAGAGGCTGTCTATGGGC
V A S L Y P R V K V Y K A S H F D I P G S A S G Y S E E R V S W A

10356 CAAGAGAAATCTCACAGAGGAGAAATACTTTCTGCTGCTCACTGACCTAGGAAGAAGAAATGTCAAGAAAGAGAGAAATATTGTCCATAATAAT
K E N S H R G E Y S L L P S S D D E E E E M S E R S E L L C H I N

10456 CAGTGTCAACAAAGCTCTTTATCTCTGGAGGACCACTGATGTCTTGGAAATGGAAGCAATGTTTGGCTCACTAAATTTGTATATTAATTTCTTA
Q C Q Q K L F Y P G G T T D V L G M E S N V W L T K F V N I K F P K

10556 AAGGAACAAAGTGATCTCTCTGATGGAAGAAATTCATAGCTGTGATCTGAGCTAAACCATATTCGAGGAATGAAATTCCTGATAGGGCAC
G T K V I L P D G R K P I A C D P E L K P L L Q E L K P L D R A T

10656 ATCTGAGTCTCTGACTCTGAAATAGAAAGCTGTAATTAACCTGATATGCACTTTGTCCAGAGTGGCAGAGTGGTATGTAATCTGTCTACTCCGGGA
S E E S D S E

10756 AAGTTTGTCTTCAATTTTCAAGACATTAAGGGTGGAAAAATATATTTCTGCTAAACCTCTGGGGACTAGAGGTGTGGAACCTTCTGCTCTCTG
CTTCAAGGGGAAGTTTTTGGTTCAATCTTTTAAAGTACTTAAAGTAAGTGAATAAATTAATCTCTCTTAAAGTATTAATGAACTATGT

10856

Fig. 2 (cont'd)

6/7

10956 ATCCTTTAAAACCATGTATTCTTTAGTCATCTAGATCTTAGAGTATGAAAAAGAAACTGCAATAGTACTATCATGTAGTAAATAAAGTACAGCTT
11056 AGTCATCTGATGATGTCTACAGAAAAGAACCTAGAGAGAGAAACAACTTTCTGCGATGCAACAGAGCGGGAGCTTGGTGTAGGAGCTAAGTCACCTCTT
11156 ACATCTAGAGCCTACTCTTCTGAACTGTTGCAATCTTAATTTTGGAACTCTTACATCACCTTTAAGAGACTGAAAAGCTGACTCTGCGCAGGAAGCT
11256 CCTTTAGGGTAGAGGAAATGTTCTCAATCTCTATCTTAAAGGGTTGCTTCATTAAAGGTTGAAACTGTGTACTGGAAGTAGATTTTSCATAACTTTAAA
11356 CTTTATAGTTGCACTGTTTCTGCTATAGCGGCTATAAAAGGGTTATGGTAGATTCTACGGGAGCTCTTCTCACAGACTTGGCTGGCTTCAGGGTGAGATT
11456 GAGACTCTCCAGCTTGGGTAAGATTCTGATGTATTTTCTGAAATATTTTGCCTTGCTCAAAATTAATAAATTGGCTTTTCTTCACTCAATTCA
11556 AGCTTCATATAATTATATTTCTGTAAGCCGAACTCACATGAGTGGTCTTCTCTATCTTGGGGAAAAGTGTCTTCTACTTGAAGTGTAGAGC
11656 TACTAAGTGAAGAACAATCTATCCCGGCTTAGGCCACGACA

Fig. 2 (cont'd)

Fig. 3

K:2978 - P 324

0988507 07868502
 07 FEB 2002

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY
 (Includes Reference to Provisional and PCT International Applications)

Attorney's Docket No.

012627-022

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

FOAMY VIRUS VECTORS FOR EXPRESSING FOREIGN GENES IN MAMMALS AND THE USE THEREOF

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application

Number _____

on _____

and was amended

on _____

(if applicable).

☒ was filed as PCT international application

Number PCT/DE99/04052

on 17 December 1999

and was amended

on _____

(if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(e) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119:

COUNTRY (If PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119
DE	198 58 441.5	17 December 1998	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

 (Application Number)

 (Filing Date)

 (Application Number)

 (Filing Date)

FULL NAME OF SOLE OR FIRST INVENTOR		SIGNATURE		DATE	
Rolf M. FLÜGEL		<i>R. Flügel</i>		August 8,	
RESIDENCE		CITIZENSHIP			
Herrmann-Brunn-Straße 43, D-69198 Schriesheim, DE		DE			
POST OFFICE ADDRESS					
Herrmann-Brunn-Straße 43, D-69198 Schriesheim, DE					
FULL NAME OF SECOND JOINT INVENTOR, IF ANY		SIGNATURE		DATE	
Martin LÖCHELT		<i>Martin Löhelt</i>		8/8/2001	
RESIDENCE		CITIZENSHIP			
Herrmann-Löns-Weg 83, D-69207 Sandhausen, DE		DE			
POST OFFICE ADDRESS					
Herrmann-Löns-Weg 83, D-69207 Sandhausen, DE					
FULL NAME OF THIRD JOINT INVENTOR, IF ANY		SIGNATURE		DATE	
R. FLOWER		<i>R.P. Flower</i>		27-9-2001	
RESIDENCE		CITIZENSHIP			
1 North Shore Hospital, Gore Hill NSW 2065, AU		AU			
POST OFFICE ADDRESS					
1 North Shore Hospital, Gore Hill NSW 2065, AU					
FULL NAME OF FOURTH JOINT INVENTOR, IF ANY		SIGNATURE		DATE	
J. WINKLER		<i>David L. Winkler</i>		23-11-01	
RESIDENCE		CITIZENSHIP			
6/28 Lampe Ave, Wagga, NSW 2650		AU			
POST OFFICE ADDRESS					
Stephen Terrace Walkerville, S.A. 5081, AU					
FULL NAME OF FIFTH JOINT INVENTOR, IF ANY		SIGNATURE		DATE	
Stephen Terrace Walkerville, S.A. 5081, AU		<i>As above</i>			
RESIDENCE		CITIZENSHIP			
POST OFFICE ADDRESS					
FULL NAME OF SIXTH JOINT INVENTOR, IF ANY					
SIGNATURE		DATE			
RESIDENCE		CITIZENSHIP			
POST OFFICE ADDRESS					
FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY					
SIGNATURE		DATE			
RESIDENCE		CITIZENSHIP			
POST OFFICE ADDRESS					
FULL NAME OF EIGHTH JOINT INVENTOR, IF ANY					
SIGNATURE		DATE			
RESIDENCE		CITIZENSHIP			
POST OFFICE ADDRESS					